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A New View of the "Synthesis of Life"

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The experimental research which is identified with our laboratory has been carried out almost entirely within the state of Florida. The credit is properly shared with numerous devoted students and talented associates. Most of what has been done was possible because a few bioscientists in the NASA office disbursed for truly basic research a fraction of the small amount available. This came from a budget which is predominantly committed to space exploration and therefore to necessarily expensive hardware. Parenthetically, I would like to state the opinion that this latter is itself insufficient to attain supremacy in space. Leadership in space is significant intellectually and societally if one believes, as do I, that the scientific secret of our ultimate origin is in the stars. Whoever tells us most thoroughly and accurately what and where man and his universe came from should be in position to tell us where man is going.

I wish also to express appreciation to a few Florida educators. Our experiments were begun in 1953 in another state in which one educational administrator admonished me not to use the "offensive" word evolution on a college television program. In contrast, I was able to name our activity at the University of Miami an Institute of Molecular Evolution, and I was attracted to the University in part by the fact that before I arrived, its catalog already frankly listed courses in evolution.

The scientific question of the synthesis of life can be analyzed as below.

$$\text{Primordial gases} \xrightarrow{\text{(Synthesis)}} \text{Small organic molecules [amino acids, N bases, etc.]} \xrightarrow{\text{(Polymerization)}} \text{Macromolecules [prebiotic protein or nucleic}$$

$$\text{acid]} \xrightarrow{\text{(Self Assembly)}} \text{Protocells} \xrightarrow{\text{(Reproduction and Darwinian Selection)}} \text{Contemporary cells and multicellular organisms}$$

We see that, in the later stages, one needs to concern himself with the evolution from a primitive organism to a contemporary unicellular or multicellular organism. This aspect of evolution is one which Darwin clarified by his selection mechanism. Looking back on the experimental research in the field of abiogenesis since 1950, I believe that the Darwinian part of the answer, explaining evolution from a first organism, represented by far the most intricate and involved aspects. That required hundreds of millions of years. When we focus our attention on the true chemical synthesis of an organism starting from nonbiological precursors, such as activatable atmospheric gases, we see that we have narrowed our questions. We have then stripped away the most forbidding part of what was not so many years ago thought of as a hopelessly imponderable problem.

The first step from primitive reactive gases to amino acids, to the nitrogen bases of the nucleic acids, or to monosaccharides represents the area in which the largest number of the few laboratories in the field have worked. Contributions have come from such laboratories as those of Calvin (1962), Ponnamperuma (1965), Oró (1965), Miller (1955), Orgel (Sanchez et al., 1966), Fox (1965, 1968), and others. The next step concerns the formation of the larger molecules, proteins, nucleic acids, and cellulose. Their formation is thought of as an appropriate type of polymerization of monomers. We can see also, by further analysis of this problem, that the following step is not one of true synthesis, but is rather one of structural organization of appropriate polymers. This kind of process has been referred to increasingly, by the biochemist, as an act of self-assembly. On this basis we should, strictly, not think and speak of the "synthesis of life", but rather of the synthesis of precursor polymers and of their self-assembly into protocells. These two steps are the ones to which we have devoted major attention (Fox, 1965). Examples of self-assembly of organelles of cells are now numerous (Seventh International Congress of Biochem., 1967). (If, in fact, one may properly employ the journalistic phrase, "secret of life", that secret may well be the power of self-assembly.)

The primitive cell, which our experiments now tell us could arise from reactant gases in less than a few hours (Fox, 1968) had then to evolve to a contemporary cell. The elegant studies that have been carried out by Goulian and Kornberg (1967) and by Spiegelman (1968) involve the dismantling of a contemporary cell and the utilization of contemporary enzymes and primer nucleic acids for further synthesis of a contemporary type of RNA or of DNA, respectively. These processes do not, therefore, answer the fundamental questions of how enzymes began in the absence of enzymes, of how cells arose in the absence of cells, or of how genes appeared in the absence of genes. Our work is aimed at these questions.

In this connection, I believe also that attempts to define life have an unscientific quality. Although the definition of life has a certain pedagogical value for beginning students, many of us who have thought about the question have come to the conclusion that life is not yet definable. The definition of life has, as Melvin Calvin (1962) stated, the quality of "subjective arbitrariness". The

TABLE 1

Catalytic activities found in proteinoids

Substrate or Reaction	Authors	Year of Publication
p-Nitrophenyl Acetate	Fox, Harada, Rohlfing	1962
p-Nitrophenyl Acetate	Noguchi, Saito	1962
p-Nitrophenyl Acetate	Rohlfing and Fox	1967
p-Nitrophenyl Acetate	Usdin, Mitz, Killos	1967
Glucose ——> glucuronic acid ——> CO_2	Fox and Krampitz	1964
ATP ——> ADP	Fox	1965
	Durant and Fox	1966
p-Nitrophenyl Phosphate	Oshima	1968
Pyruvic acid ——> acetic acid + CO_2	Krampitz and Hardebeck	1966
	Hardebeck, Krampitz and Wulf	1968
Oxaloacetic acid ——> pyruvic acid + CO_2	Rohlfing	1967
Amination of α-ketoglutaric acid	Krampitz, Diehl, and Nakashima	1967
	Krampitz, Baars-Diehl, Haas, and Nakashima	1968
Dehydrogenation of glutamic acid	Krampitz, Haas, Baars, and Nakashima	1968

facts are not yet entirely at our disposal, at least not in a way that life scientists agree that they are. Any definition is a kind of judgment, so that one who renders a definition of life, i.e. a judgment, is making the judgment before having the facts. Accordingly, I consider such definitions to be unscientific.

Our research is centered around the production of polymers of amino acids by simple heating under conditions which are not only imputable to the primitive Earth but are wide-spread on the contemporary Earth.

These protein-like polymers, or proteinoids have been shown in six laboratories to have many kinds of catalytic activity for natural substrates (Table 1). We are thus able to visualize how the first enzymes could have arisen in the absence of enzymes to make them. The appropriate geological environment and diverse amino acids would have been sufficient.

A somewhat unexpected characteristic of the proteinoids is that they represent quite highly ordered polymers (Fig. 1). The order

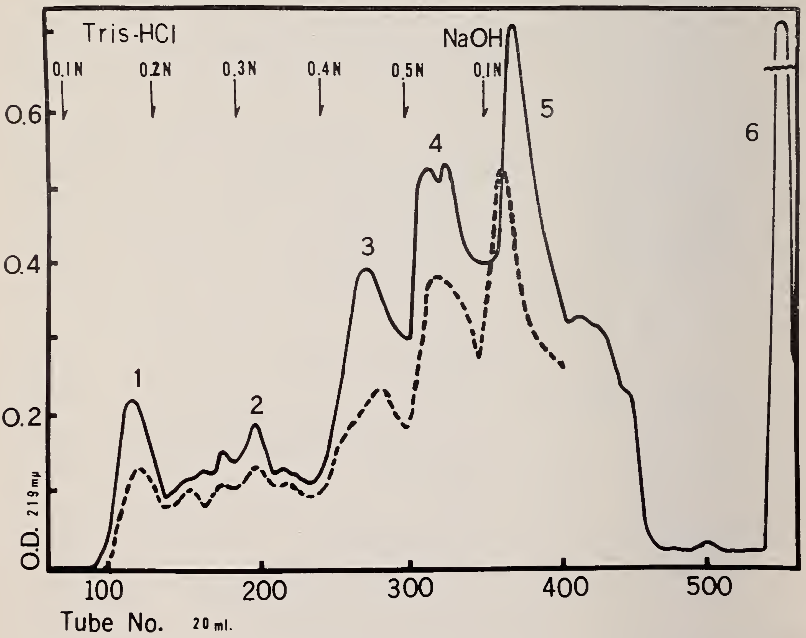


Fig. 1. Elution pattern of 1:1:1-proteinoidamide fractionated on DEAE-cellulose.

results internally from the selective interaction of the amino acids which are heated. We are able to understand this effect as due to the special shape and distribution of charge of each of the eighteen kinds of reactant amino acid. The evidence for this great limitation in heterogeneity has been published recently (Fox and Nakashima, 1967). A principal significance of such results is that they suggested that prebiotic proteins might first have come into existence in the absence of nucleic acids to order the sequences in those first proteins (Fox, 1965). This suggestion has also been made more recently by Steinman (1967) working with amino acid reactions in simpler systems. While prebiotic protein need not have had all of the properties of contemporary protein, it would have had to have sufficient to begin the line, e.g., macromolecular order, metabolism, and cellular structure (Table 2).

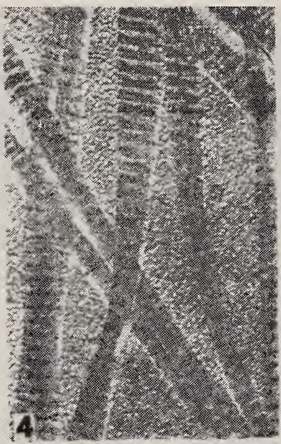
We may turn now in more detail to the question of self-assembly and the origin of the cell. In 1954 Professor George Wald (1954) wrote, "For a time this problem of molecular arrangement seemed

TABLE 2

Properties of thermal proteinoids in common with those of contemporary proteins. Fox (1965) and bibliography.

Limited heterogeneity
Qualitative composition
Quantitative composition
Range of molecular weight
Color tests
Solubilities
Inclusion of nonamino acid groups
Optical activity
Salting-in and salting-out properties
Precipitability by protein reagents
Hypochromicity
Infrared absorption maxima
Recoverability of amino acids on hydrolysis
Susceptibility to proteolytic enzymes
Catalytic activity
Inactivatability of catalysis by heating in aqueous solution
"Nonrandom" (nonuniform) sequential distribution of residues
Nutritive quality
Morphogenicity

to present an almost insuperable obstacle in the way of imagining a spontaneous origin of life, or indeed, the laboratory synthesis of a living organism. It is still a large and mysterious problem, but it no longer seems insuperable. The change in view has come about because we now realize that it is not altogether necessary to bring order into this situation; a great deal of order is implicit in the molecules themselves." This concept of the specification of morphology by the nature of a precursor macromolecule is an extrapolation of the concept, now experimentally supported, that order in primitive proteins was first determined internally by the reacting amino acids. Professor Francis Schmitt (1956) first demonstrated self-assembly of fibrils of the protein collagen (Fig. 2). Dr. A. I. Oparin has done just one kind of experimentation in the field which he first called the origin of life, and for which I now prefer the nineteenth century phrase of spontaneous generation. Oparin used coacervate droplets (Fig. 3) made from gelatin



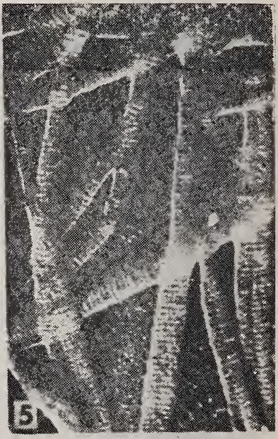


Fig. 2. Electron micrograph of microfibrils assembled from collagen, Figs. 2, 4, 5. From Schmitt (1956).

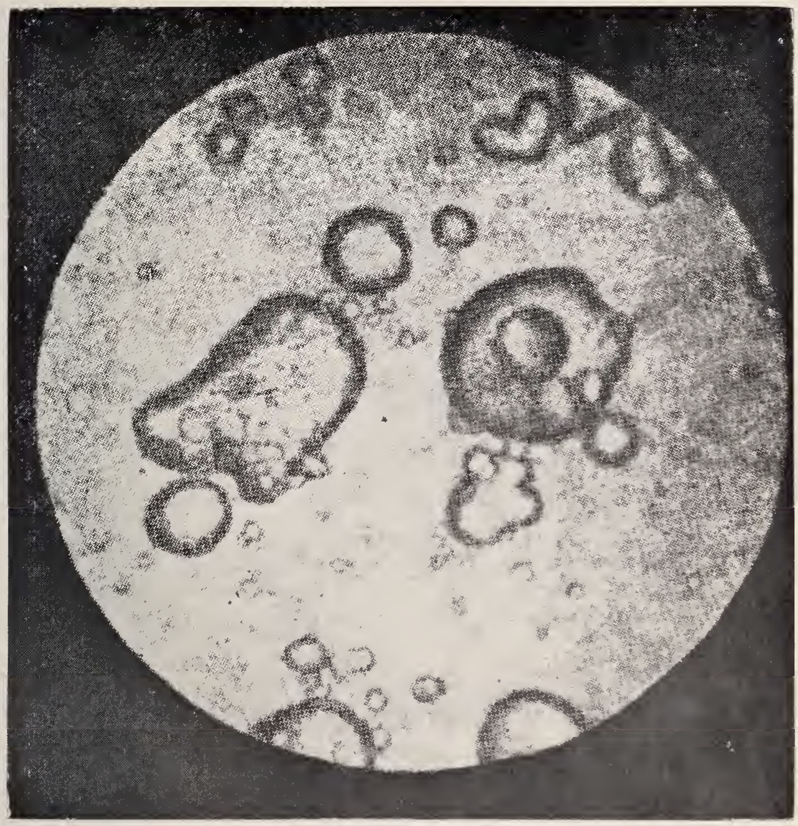


Fig. 3. Coacervate droplets. From Oparin.

(1965) as models of self-ordering, or self-assembly, of the first cell. Among the defects, which Oparin acknowledges, this model employs polymers from living things. As in other experiments, these fail to answer the question of how cells arose when there were no cells to produce them. This question is now answered in principle by the proteinoid, in a process so simple that it resembles that of making instant coffee.

When hot water is poured onto proteinoid, and the resultant clear hot solution is cooled, millions of microscopic spherules separate (Fig. 4). These are stable to centrifugation, they have a kind of osmotic property, they can be made gram-negative or gram-positive, they have catalytic powers, they can be produced so that

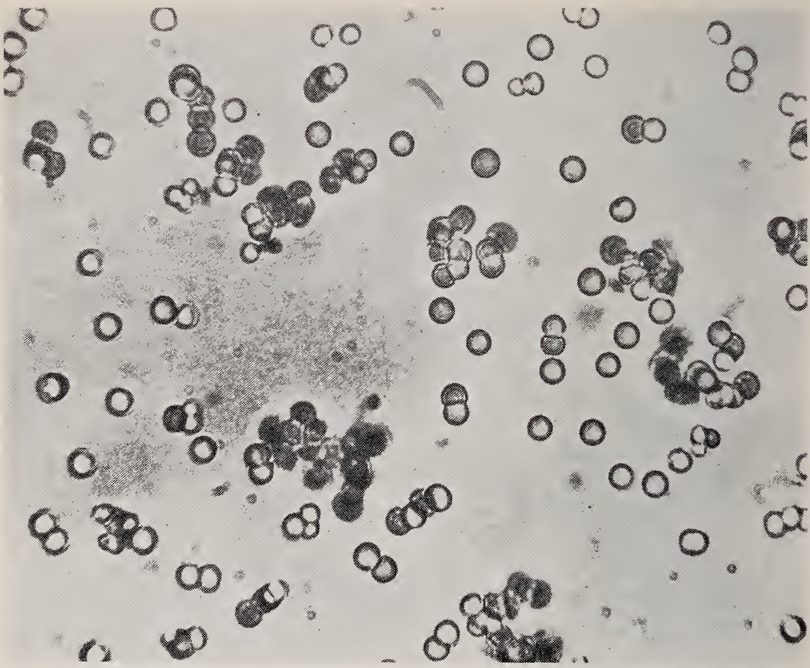


Fig. 4. Proteinoid microspheres. Approximately 2μ in diameter.

they are motile, they bind polynucleotides as well as dyes, and they also show some selectivity in the passage of molecules through their boundaries (Fig. 5).

As Fig. 6 shows, these boundaries are structured. One may in this figure compare a section of a proteinoid microsphere with one of *Bacillus cereus* under the electron microscope. Experts who are uninformed on these units often guess wrong as to which is which, reportedly because the artificial particle has a thicker boundary. In the same figure we see that the artificial boundary is a double layer. This has permitted some broadening of our understanding of the Danielli model (1935) of the unit membrane of the contemporary cell, especially with regard to the contribution of lipid.

In Fig. 7, we observe a cyclic phenomenon which is intrinsic to the units composed of proteinoid. In the first picture are shown microspheres which have, during a week in their liquor, developed buds which in appearance, texture, and tenacity re-

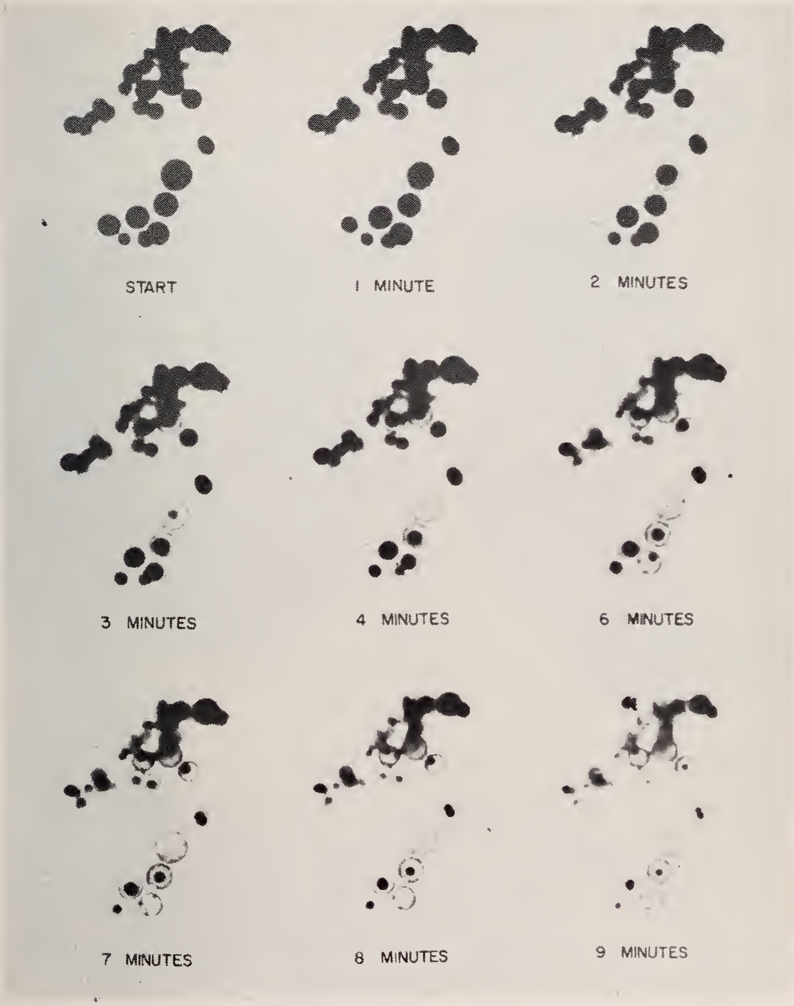


Fig. 5. Time-lapse study in ultraviolet light of diffusion of polymer outward from microsphere when pH is raised slightly. Experiment performed by Mr. R. J. McCauley with Dr. Philip O'B. Montgomery.

semble buds on yeast. In the second photomicrograph, the buds have been removed, a phenomenon resulting from heat, electrical, or mechanical shock. These are then stained with Crystal Violet and tranferred to a solution of proteinoid saturated at 37° and allowed to cool to 25° over one hour. The buds grow by a kind of heterotrophic process. In the last picture, one can see one of the microspheres with a second generation bud. In this manner, we can visualize an evolution from simple physical processes acting on

Fig. 6. Electron micrographs showing section of *Bacillus cereus* in upper left. Section of proteinoid microsphere in upper right. Lower micrograph displays double layer in boundary of proteinoid microsphere treated with buffer to raise the pH of a suspension.

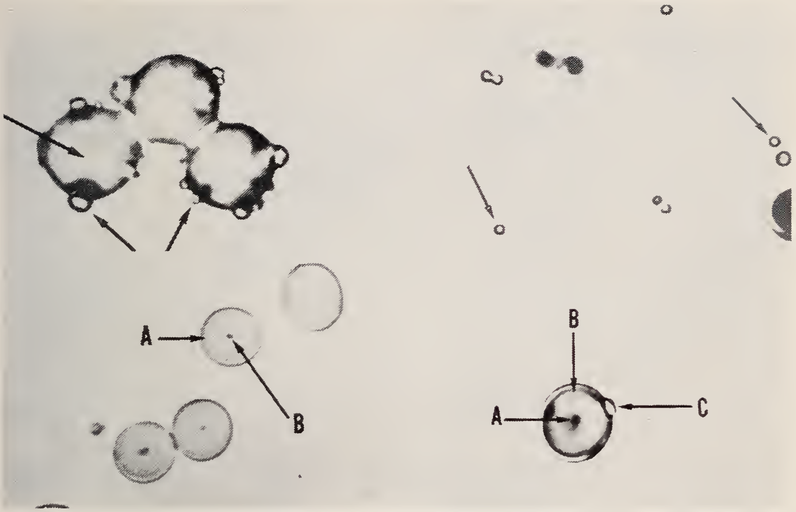


Fig. 7. Optical micrograph of proteinoid microsphere replicating by budding and heterotrophic growth. a) Microspheres with buds, b) Buds after removal, c) Microspheres which have grown from stained buds, d) Microsphere with second generation bud.

simply derived polymers to yield the minimal complexity required for reproduction.

While these experiments have not produced a fully contemporary type of organism, they have shown how a proteinaceous microparticle with internally ordered macromolecules, catalytic activities, and many of the properties of a contemporary cell, including the ability to participate in a presumably primitive reproductive process can, and could be, spontaneously produced. The necessary geophysical conditions, being found in abundance on the contemporary Earth (Fox, 1966), should have earlier been abundant also. This rugged process to the primordial stage can be visualized as having occurred quickly, easily, and often when amino acids with a small proportion of aspartic acid, glutamic acid, or lysine were present.

Without yet defining life, we can use this physical model to help describe life. The model suggests that life is a range of associations of unique chemical materials having intrinsic and characteristic physical properties. Many or all of these properties have their simple physical counterparts. In the growth of buds removed from microspheres, for example, we see a similarity to the growth of inorganic crystals. But these units are not inorganic crystals; they are composed of organic material which has an array of catalytic activities such as to what would be needed biochemically in the evolution of metabolism. We are thus dealing with complex macromolecules, and especially with supramolecular organization or systems. These systems have properties of association, differentiation, and other behavior which are simply not to be found at the more rudimentary or molecular level.

The model answers in one way how cells could arise in the absence of cells, how enzymes could come into existence in the absence of enzymes to make them, and how macromolecular information could arise in the absence of nucleic acid. Experiments demonstrating one type of origin of primordial ribonucleic acid have been published from our laboratory. Based on these studies, Carl Woese (1968) has reported experiments constructed to explain the origin of the code between RNA and protein, and with Dr. Waehneldt we also have other reports of selective interaction of RNA, DNA, and proteinoid (Waehneldt and Fox, 1968). The idea that protein first arose before or with RNA and DNA is not a new suggestion; many theorists, e.g. Lederberg (1961), Thimann (1965), have advanced this conceptual possibility in the past. What is a new view is the detailed interpretation of our physical model consistent with that suggestion.

Among studies under way are attempts at further contemporization of the proteinoid microsphere, especially through incorporation of internal mechanisms for synthesis of biopolymer, polyamino acids, and coding polynucleotide. At least two laboratories are studying the potentialities of proteinoids as food. At least two laboratories are studying the relationship of pyrolysis of amino acids to the origin of petroleum. We believe we have seen in the properties of microspheres clues to the study of models of behavior at the most primitive evolutionary level, as in chemotaxis. Some of the data obtained on the proteinoids are being interpreted in a context which is more fundamental than that of the origin of life, the relationship of entropy to general evolution. Needless to say, what has been done so far has helped to discipline plans in the search for extraterrestrial life (Fig. 8). Here we see compari-

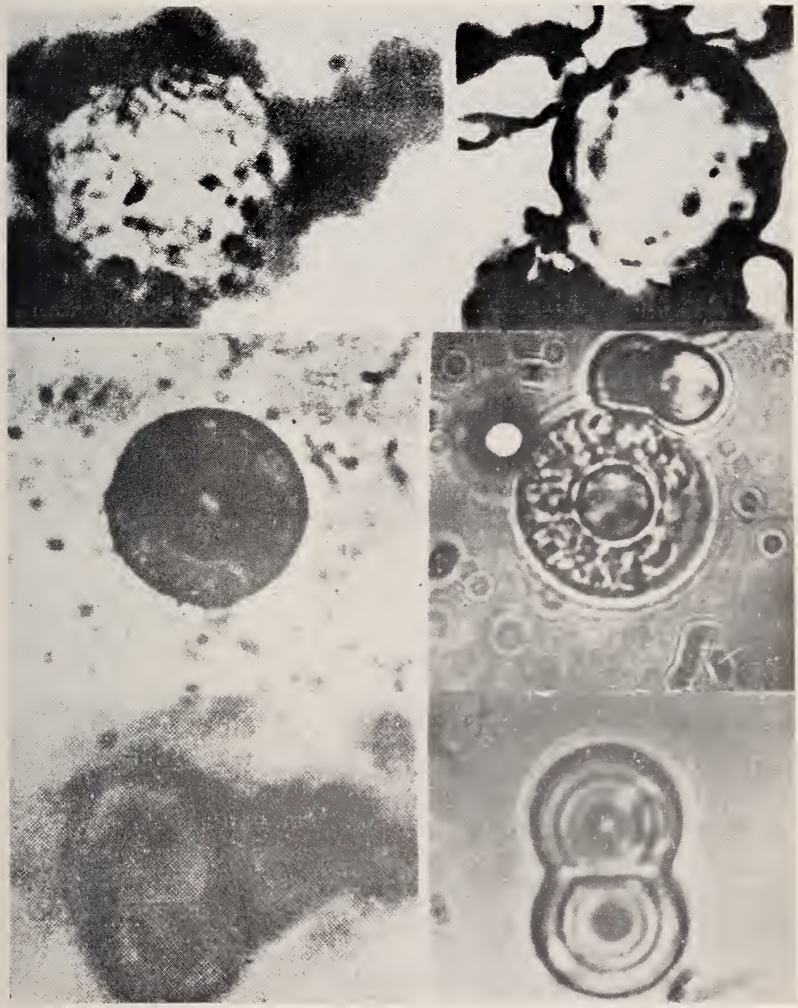


Fig. 8. "Organized elements" of carbonaceous chondrites (left) and proteinoid microparticles (right).

sons of "organized elements" from a meteorite with proteinoid microparticles. These have been much republished.

Among other consequences, this study has focused attention on what the Space Research Committee at the University of Miami has titled simply *The Survival of Man.* Our space research group is concerned with identifying the problems and possible solutions of the survival, terrestrial and extraterrestrial, of man as he seemingly hurries to his own destruction. This is a somber note to end on, but not necessarily a pessimistic one. Anyone who has a curiosity about and a reverence for life, I believe, must be concerned with how it began and must also be concerned with doing what can be done to insure its continued and internally controlled evolution. In many ways, some of them indirect, study of the synthetic origins of life leads into studies of its maintenance and preservation.

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